lethal GVHD while maintaining GVL activity after BMT with allogeneic naive (uncultured) thymidine kinase (TK)-expressing transgenic T cells. However, the efficacy of GCV control of GVHD and its impact on GVL activity following BMT with ex vivo activated, TK transduced and selected (Td) donor T cells has not been thoroughly characterized because of the significantly reduced GVHD-inducing potential of cultured cells compared to naive T cells in both preclinical models and initial clinical trials. In these experiments, we used A20 tumor cells (B cell lymphoma of BALB/c origin) that were modified to stably express a click beetle red luciferase-EGFP fusion reporter gene (A20-CBRluc/egfp) and monitored disease progression in vivo using optical bioluminescence imaging (BLI). To induce leukemia, BALB/c recipients were lethally irradiated and reconstituted with C57BL/6 BM supplemented with or without A20-CBRluc/egfp leukemia cells. Mice receiving leukemia cells were then left untreated or injected with 4×10^6 ΔCD34-TK (chimeric fusion suicide gene) Td donor T cells the day of BMT. Ten of ten BALB/c mice transplanted with BM and A20-CBRluc/egfp cells exhibited tumor engraftment and growth, with 8 mice dying from leukemia before day 35. In contrast, we observed no significant tumor signal in the majority (92%) of untreated (No GCV) mice that received ΔCD34-TK Td T cells. However, these animals developed GVHD with high mortality ($P = .003$ compared to BM only control) and significant weight loss ($P < .05$ from day 10 onward compared to BM only control). Although mice treated with GCV from days 4–10 after BMT were protected from GVHD, 50% of these animals developed leukemia. In contrast, none of the mice developed leukemia if GCV treatment was delayed until day 10 after BMT. Importantly, this GVL effect was obtained in the absence of GVHD, as evidenced by the improved survival (88% survival at day 60 post-BMT) and weight gain of the day 10–16 GCV treated mice compared to the untreated (No GCV) control. These data suggest that donor T cell dependent GVL effects against A20 cells can be preserved while the severity of GVHD is reduced, further demonstrating the potential of the HSV-TK/GCV system to reduce acute GVHD without impairing GVL activity.

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"MINI" METHOTREXATE AND FK506 AS GVHD PROPHYLAXIS IN PEDIATRIC UMBILICAL CORD BLOOD TRANSPLANTATION

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A majority of umbilical cord blood transplants to date utilize steroids and cyclosporine for graft-versus-host disease (GVHD) prophylaxis. Steroids are associated with multiple complications including hyperglycemia, hypertension, avascular necrosis/osteoporosis, and significant risk of infection. In an effort to decrease the adverse effects of GVHD prophylaxis, we chose an alternative combination of "mini" methotrexate (5 mg/m^2 × 4 doses) and FK506. Here we report our experience with this GVHD prophylaxis regimen. Ten children (ages 4 months to 13 years) underwent umbilical cord blood transplant at the University of Florida between June 2004 and June 2005. All patients received a cord blood unit that was matched at 6/6 (n = 4), 5/6 (n = 2), or 4/6 (n = 4) HLA loci. Children with malignant disease received either Cyclophosphamide/TBI or Etoposide/TBI as the preparative regimen, while children with non-malignant disease received Busulfan, Cyclophosphamide, and ATG. Umbilical cord blood cell dose ranged from 3.4–23.7 (median 7.8) × 10^{7}nucleated cells/kg. All children received Methotrexate 5 mg/m^2 on days 1, 3, 6, and 11, with Leucovorin on days 4, 7, and 12. No doses of Methotrexate were held. FK506 was started as a continuous infusion at day −3, targeting levels of 10–15. The FK506 was changed to twice daily oral dosing when tolerated. **Results:** 8 of the 10 children engrafted, with engraftment occurring at a median of 15 (range 13–38) days post transplant. Rates of acute and chronic GVHD were low, with no grade III or IV acute GVHD seen, and no extensive chronic GVHD occurring. Two children had grade II acute GVHD (skin and GI). Two children developed limited chronic GVHD of the skin while weaning FK506. GHVD resolved completely in these patients with an increase in FK506 and were subsequently were weaned off immunosuppression. Mucositis was very similar to other GVHD regimens. One child became CMV PCR positive, with CMV on bronchoscopy, however had mild disease (never required oxygen) and recovered completely. There were otherwise no serious infections in this patient population beyond the time of engraftment. FK506 and "mini" Methotrexate for GVHD prophylaxis in pediatric patients undergoing umbilical cord blood transplant is well tolerated. Engraftment was not delayed and mucositis was not severe. Low rates of GHVD were seen with minimal serious infections, making this an attractive option for GVHD prophylaxis.

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SUCCESSFUL TREATMENT OF REFRACTORY GVHD IN AN INFANT WITH MALIGNANT OSTEOPETROSIS WITH A HUMAN ADULT MESENCHYMAL STEM CELL BASED DRUG

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It has been suggested that adult human mesenchymal stem cells (hMSCs) have a potential to treat graft-versus-host Disease (GVHD). At the present time, Osiris Therapeutics is in clinical development with Prochymal™, a cellular therapy consisting of allogeneic bone marrow derived adult hMSCs for the treatment of acute gastrointestinal GVHD. The present work reports a successful treatment of an 8-month-old infant with grade III skin and gut GVHD using Prochymal™. The infant was born with hypocalcemia with normal phosphorus and carbonic anhydrase II, and elevated PTH. Skeletal survey and bone marrow biopsy were consistent with osteopetrosis. The infant received a 4/6 unrelated cord blood transplant after standard Busulfan/Cyclophosphamide/ATG conditioning. The first graft failed and the patient developed autologous reconstitution. A second transplant (4 of 6 matched cord blood) was given at 5 months of life after Fludaribine/Cyclophosphamide/ATG conditioning regimen. The patient developed severe acute grade III skin GVHD with gastrointestinal involvement in the form of bloody diarrhea that was resistant to high dose steroids, Tacrolimus, Cellcept, and anti-CD25 antibody. A compassionate use protocol was developed for the treatment of the patient with Prochymal™. Two intravenous infusions of Prochymal™ were given at a dose of 8 × 10^6 cells/kg body weight each 3 days apart starting on day 100 post transplant. Both infusions were well tolerated with no associated toxicity or reaction. By the fourth day of treatment, there were initial signs of improvement of the skin. By day 7, the skin improvement was dramatic and episodes of diarrhea had ceased. By day 14, the resolution of this patient's refractory GVHD was essentially complete. Grade IV skin GVHD recurred approximately 1 month following initial treatment after weaning of immune suppression. Repeat treatment with Prochymal™ was administered starting on day 120 post transplant and GVHD symptoms diminished within 1 week. The patient developed 100% donor chimerism. The patient's myeloid engrafted improved from 8% on day 105 post transplant to 100% by day 145. Data suggest that MSCs suppressed the adverse immunological response, healed damaged skin and gut tissue, and aided in engraftment. We conclude that Prochymal™ may be clinically useful for treatment of refractory cases of GVHD.

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SERUM LEVELS OF SOLUBLE INTERLEUKIN-2 RECEPTOR IS A POWERFUL MARKER OF ACUTE GRAFT-VERSUS HOST DISEASE AFTER HLA-HAPLOIDENTICAL BONE MARROW TRANSPLANTATION

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To examine whether serum levels of soluble interleukin-2 receptor (sIL-2R) is a good marker of acute graft-versus-host disease (aGVHD), they were measured in 37 patients receiving HLA-haploidentical bone marrow transplantation (BMT). Grafts were from HLA-haploidentical relatives. Conditioning regimen was myeloablative including cyclophosphamide and toral body irradiation. GVHD prophylaxis included tacrolimus (TAC)/solumedrol (mPSL)/methotrexate (MTX) (n = 11) and tacrolimus (TAC)/solumedrol (mPSL)/methotrexate (MTX)/mycophenolate mofetile (MMF) (n = 27). Seventeen patients developed aGVHD (Grade I, 9; Grade II, 5; Grade III, 3) after BMT. There was a significant correlation between occurrence of a GVHD and the maximal serum level of sIL-2R. For 11 patients receiving GVHD prophylaxis with TAC/mPSL/MTX, aGVHD occurred in 7 patients (63%). The mean maximal level of sIL-2R (±SE) in 7 patients with a GVHD was 7086 (±1066) and that in 4 patients without aGVHD was 2770 (±450). For patients with GVHD prophylaxis with TAC/mPSL/MTX/MMF, aGVHD occurred in 10 patients (37%). The mean maximal level of sIL-2R in 10 patients with aGVHD was 3944 (±655) and that in 17 patients without aGVHD was 2566 (±358). These data suggest that serum levels of sIL-2R are useful for predicting the occurrence of aGVHD after HLA-haploidentical BMT.

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EVALUATION FOR T CELL POPULATIONS AND CYTOKINE SHIFTS IN NEWLY DIAGNOSED PEDIATRIC CHRONIC GRAFT-VERSUS-HOST DISEASE

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Little is known regarding the immunopathogenesis of chronic graft-versus-host disease (cGvHD) in children. From 2001 through 2005, the Children's Oncology Group (COG) performed a randomized, double blinded, placebo controlled phase three study for newly diagnosed extensive cGvHD in children and adolescents, ASCT0031. Based on murine model data and limited human studies, we hypothesized that cGvHD would be characterized by a Th1/Tc1 shift in T cell activation. Correlative immunologic studies were performed on patients at time of enrollment to evaluate for immune cell populations and T cell function, including cytokine production. Twenty-seven control patients without evidence of cGvHD had peripheral blood collected at 3, 6, 9, and 12 months post hematopoietic stem cell transplant (HSCT) and were used as time matched controls. Fifty-four patients with newly diagnosed extensive cGvHD were evaluated and divided as early cGvHD (3–5 months post HSCT; N = 20) or late-onset cGvHD (≥6 months post HSCT; N = 34). Significance was determined by either a 50% increase or decrease of the mean value compared to control with a P value ≤ .05. In the early-onset cGvHD patients, the total number of CD3+, CD4+, and CD8+ T cell subsets were all lower relative to non-cGVHD controls, although none were significantly different. In the late onset cGVHD patients, a non-statistically significant increase in CD4+ and CD8+ T cell populations were noted compared to time-matched controls. Functional evaluations for cytokine production after in vitro PMA/Ionomycin stimulation (intra-cytoplasmic staining by FACS) revealed no significant difference in either Th1 cytokine production (IFNγ) by CD4+ or CD8+ T cells with early-onset (P = .82, 0.81, respectively) or late onset cGVHD (P = .40, P = .33, respectively). Evaluation for a Th2/Tc2 shift revealed that IL-4 in either CD4+ or CD8+ T cells at early-onset cGvHD (P = .35, 0.11, respectively) or late-onset cGvHD (P = .54, P = .64, respectively) was not different. Thus, we were unable to demonstrate either a Th1/Tc1 or Th2/Tc2 shift associated with newly diagnosed cGvHD in children. These results may be different to previous adult HSCT studies in that there may be a difference in the biology of chronic GvHD in younger HSCT recipients or that these studies were limited to evaluation at the time of presentation of disease.

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BRONCHIOLITIS OBLITERANS AND BRONCHIOLITIS OBLITERANS ORGANIZING PNEUMONIA IN PEDIATRIC ALLOGENEIC STEM CELL TRANSPLANT: AN INSTITUTIONAL EXPERIENCE

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Bronchiolitis obliterans (BO) and bronchiolitis obliterans organizing pneumonia (BOOP) are rare, well-recognized complications of allogeneic stem cell transplant (SCT). Both BO and BOOP are associated with high morbidity and mortality in adult patients. However, in the pediatric population, the incidence and morbidity of these complications have not been well described. We report our institutional experience of BO and BOOP in 449 pediatric allogeneic SCT patients between January 1, 1993 and December 31, 2004. The source of stem cells in this population was as follows: 200 unrelated donors, 198 siblings, 42 parents, 5 extended family members, and 4 half-siblings. A total of 18 patients (4%) developed BO (n = 11) or BOOP (n = 7) during this time. The diagnosis of BO was based on pulmonary function test (PFT) abnormalities (decline in FEV1 ≥20% of baseline or FEV1/FVC <70%) or characteristic histologic changes on lung biopsy. BOOP was diagnosed by pathology only. The most common conditioning regimen was cyclophosphamide (Cy) and total body irradiation (TBI), used in 12 patients. Cytarabine/Cy/TBI was used in 3 cases, Busulfan/Cy in 2, and 1 patient received Etoposide/Cy. All but 2 patients received bone marrow as a stem cell source; the remaining patients received peripheral blood stem cells. Three of the patients received stem cells from fully matched related donors; 6 of the donors were related, but matched at less than 6 of the 6 typed HLA loci. Nine patients received fully matched unrelated donor transplants. The median time to diagnosis of BO or BOOP was 329 days from stem cell infusion. Ten patients had abnormal computed tomography scans at diagnosis. Six patients were diagnosed by PFTs alone, 9 by pathology alone, and 3 by PFTs and pathology. All patients were treated with immunosuppression: 16 patients received corticosteroids, 15 patients received calcineurin inhibitors, and 2 patients received mycophenolate mofetil. Azithromycin was used in 3 patients for anti-inflammatory effects. Four patients were treated with other medication regimens. One patient (6%) experienced complete resolution of pulmonary disease. Three patients (17%) achieved partial resolution. Four patients (22%) had progressive disease. Ten patients (56%) died; 7 of pulmonary disease and 3 of unrelated causes. BO and BOOP, while uncommon, are associated with considerable morbidity and mortality in pediatric SCT, and new therapeutic modalities are needed to improve the outcome of these patients.

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CELLCEPT/CYCLOSPORINE AS PROPHYLAXIS AGAINST GRAFT- VERSUS-HOST DISEASE IN PEDIATRIC PATIENTS UNDERGOING ALLOGENEIC STEM CELL TRANSPLANTATION

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Traditionally, pediatric patients are prophylaxed against acute graft-versus-host disease (GvHD) with cyclosporine combined with either methotrexate Mtx) or methylprednisolone (Pred). Mtx worsens the severity of mucositis and renal insufficiency while pred causes muscle wasting, hypertension and increased infections. Cellcept (myophenolic acid) is a better tolerated, less toxic agent with synergistic immunosuppressive effects when combined with cyclosporine or tacrolimus. We explored its use in combination with cyclosporine in 21 pediatric patients undergoing allogeneic transplantation. Patients with both malignant (n = 7) and non-malignant (n = 14) conditions ranging in age from 2 weeks to 15 years were transplanted after myeloablative preparative regimens with either related bone marrow or cord blood (n = 7) or unrelated cord blood (n = 14), using cellcept and cyclosporine for GVHD prophylaxis. Cellcept and cyclosporine were administered intravenously through the first 40-60 days post transplant using cellcept at a dose of 15mg/kg/dose IV q8H beginning on day -2 or -3. The patient then transitioned to oral therapy at the same dose and